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Title: High rates of neurodevelopmental risk CNVs in patients with intellectual disabilities and co-morbid psychiatric disorders

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Abstract

Background: Copy number variants (CNVs) are established risk factors for neurodevelopmental disorders (NDD). To date the study of CNVs in psychiatric illness has focused on single disorder populations. The role of CNVs in individuals with intellectual disabilities (ID) and psychiatric co-morbidities are less well characterised.

Aims: To determine the type and frequency of CNVs in adults with ID and co-morbid psychiatric disorders.

Method: Chromosomal microarray analysis of 599 adults recruited from ID psychiatry services at three European sites.

Results: The yield of pathogenic CNVs was high – 13%. Focusing on established NDD risk loci we find a significantly higher frequency in ID and co-morbid psychiatric disorder (10%), compared with healthy controls (1.2%, $p<0.0001$) schizophrenia (3.1%, $p<0.0001$) and ID/autism spectrum disorder (6.5%, $p<0.00084$) populations.

Conclusions: In the largest sample of adults with ID and co-morbid psychiatric disorders to date, we find a high rate of pathogenic CNVs. This has clinical implications for the use of genetic investigations in ID psychiatry.

Declaration of interest: None.

Introduction

Neurodevelopmental disorders (NDDs) are a group of disorders that are characterised by perturbed neurodevelopment — intellectual disabilities (ID), autism spectrum disorders (ASD) and schizophrenia are all considered to be NDDs (1). A proportion of the risk for NDDs can be attributed to a class of genetic variants known as copy number variants (CNVs) (2). A CNV is typically defined as a segment of DNA >1 kilobase, which is present at a higher (duplication) or lower (deletion) copy number as compared to a reference genome (3). ID has its onset in childhood and initially manifests with failure to meet developmental milestones, known as developmental delay (DD). In adulthood, a clinical diagnosis of ID is typically given when there are both deficits in adaptive and intellectual functioning (IQ score <70). ID can occur in isolation or in combination with a range of somatic, psychiatric and behavioural disorders. Association studies have shown the involvement of CNVs in psychiatric risk, in particular CNVs have been strongly implicated in the aetiology of schizophrenia (4) and ASD (5). Furthermore, investigations in large paediatric cohorts have revealed CNV regions that are significantly associated with ID (6). Many of these CNVs operate across traditional diagnostic boundaries: for example, 11 of the CNVs associated with ID are also risk factors for schizophrenia (7). The NDD risk CNVs that have been identified to date confer moderate to large risk (Odds Ratio 1.5 — ≥50)(7), and therefore have important clinical implications for affected individuals and at risk family members.

A major challenge in the clinical interpretation of CNVs is the variable penetrance and expressivity of many NDD risk CNVs. For example not all individuals with a particular CNV display a neurodevelopmental phenotype (penetrance) and not all individuals express a severe phenotype (expressivity) (8). A large proportion — approximately 50% — of adult ID is idiopathic or of unknown cause (9). Chromosomal microarray analysis (CMA), the group of tests used to detect CNVs, have been one of the recommended first-tier test for clinical investigation of idiopathic ID since around 2010 and have primarily been undertaken in paediatric populations (10). Testing of adults with ID is particularly important for elucidating the relationship between rare CNVs and late-onset medical and psychiatric phenotypes. Indeed, the highest burden of pathogenic CNVs may be present in adults expressing co-morbid neurodevelopmental phenotypes.

Method

To the best of our knowledge, this study is the first multi-population analysis of CNVs in adults with ID and psychiatric co-morbidities and represents the largest sample of its kind to date. We aimed to determine; (i) the frequency of known NDD risk CNVs as compared to large population cohorts from the literature (healthy controls, ID/ASD and schizophrenia) (7); (ii) the overall rate of pathogenic CNVs; (iii) the relationship between pathogenic CNVs, level of ID and co-morbid psychiatric diagnoses; and (iv) likely pathogenic CNVs affecting neurodevelopmental candidate genes.

Participant recruitment

The GENMID (GENetics of Mental disorders in Intellectual Disability) consortium is comprised of three primary research groups based in Catalonia, Spain; Leuven, Belgium; and England, United Kingdom. In Catalonia participants were identified between 2009 — 2012 from the Mental Health ID regional community Service Parc Hospitalari Martí i Julià, Girona. In Leuven, participants were recruited between 2005 — 2015 at the regional inpatient psychiatric unit for adults with ID in the St-Camillus Psychiatric Hospital, Bierbeek. Initially, only patients diagnosed with psychosis were recruited, but recruitment was later extended to other psychiatric phenotypes. In England, participants were recruited by consultant psychiatrists in intellectual disabilities between 2012 — 2015 from ID psychiatry caseloads at 32 National Health Service (NHS) trusts and 1 non-NHS provider. Written informed consent was obtained for all participants with capacity to consent and consultee/guardian advice was sought in absence of this.

Recruitment criteria

All sites recruited adults over the age of 18 years. Participants had idiopathic ID, defined as no clear genetic or environmental cause of ID as detailed in their medical records. Participants had one or more co-morbid psychiatric diagnoses and/or significant challenging behaviours.

Phenotypic assessments

For all sites the ID levels are in accordance with the ICD-10 ranges (<20 profound ID, 20—34 severe ID, 35—49 moderate ID, 50—69 mild ID, 70—84 borderline ID). For further

analyses, the <20—49 ranges were collapsed into a severe category and the 50—84 ranges were collapsed into a mild category. All sites identified psychiatric diagnoses from medical records and/or informants. Psychiatric diagnoses were converted from Diagnostic and Statistical Manual of Mental Disorders IV to ICD-10 criteria (with agreement between two psychiatrists).

Genetic analysis and CNV Calling

DNA was extracted from blood and saliva samples. Samples from Catalonia were analysed using the 400K Agilent platform (Agilent Technologies, Santa Clara, California, USA) at the Genetics Laboratory, UDIAT-Centre Diagnòstic, Parc Taulí Hospital Universitari. Samples from Leuven were analysed on the CytoSure ISCA oligoarray set (OGT, Oxford, UK) at the Constitutional Cytogenetics Unit of the Center of Human Genetics. Samples from England were analysed on the NimbleGen 135K platform (87%) (Roche NimbleGen, Madison, Wisconsin, USA) and the Cytoscan 750K platform (13%) (Affymetrics, Santa Clara, California, USA) at the North East Thames Regional Genetics Service Laboratory.

CNV calling took place at the respective clinical laboratories, in keeping with internal laboratory protocols based on the American College of Medical Genetics best guidelines (11) or the Association of Clinical Genetic Science Best Practice Guidelines (12).

CNVs reported by the clinical laboratories were classified into three categories: pathogenic, uncertain clinical significance and benign. The genome coordinates for all sites are reported according to the National Center for Biotechnology Information (NCBI) human genome build 37 (hg19, February 2009). All pathogenic CNVs were fed back to the participants' treating psychiatrist.

Analysis methodology

We aimed to compare the rates of known rare NDD risk CNVs in our cohort to rates in ID/ASD and schizophrenia cohorts. We used a list of 63 NDD risk CNVs that were associated with ID (6) and or schizophrenia from Rees *et al.* (7), henceforth referred to as NDD CNVs. NDD CNV carriers were identified using the criteria outlined in Kendall *et al.* (13), also used by Rees *et al.* (7), (personal communication), see supplementary table 1.

CNVs fulfilling these calling criteria were classified as pathogenic and are included in the diagnostic yield. Duplications or deletions of the same chromosomal region were counted as separate loci (e.g. 22q11.2 del and dup). A rate percentage was calculated to enable comparisons between different sample sizes and chi-square tests were used to determine the population differences. The significance level has been adjusted to p=0.01 to account for multiple pairwise comparisons.

To determine the CMA yield each individual was grouped by the most pathogenic CNV detected. Between site discrepancies were reclassified in accordance with Kearney *et al.* (11), see supplementary table 2. CNVs designated as of uncertain clinical significance were reclassified into likely benign or likely pathogenic using this methodology. We examined all likely pathogenic CNVs for recurrence and describe the main loci that have been implicated as NDD risk factors in the current literature.

Finally, we performed chi-square tests (or Fisher's exact were there were five or less individuals) to examine the differences between psychiatric diagnoses, ID level and CNV pathogenicity. Since many of the co-morbid diagnoses are correlated and thus are non-independent, correction of p-values through Bonferroni or other methods was deemed too stringent. Thus, we present all p-values uncorrected for multiple testing as recommended by several authors (14,15), while indicating the number of tests performed if all comparisons are not presented. All analyses were performed using R version 3.3.1 (16).

Results

We recruited 599 adults (Catalonia (n=80), Leuven (n=272) and England (n=247)) with ID and one or more co-morbid psychiatric diagnoses/challenging behaviours (376 (62.8%) male, mean age 43.2). Just more than half of the sample (50.8%) had severe ID and the remainder had mild ID. Each participant had on average 1.6 co-morbid psychiatric diagnoses, with pervasive developmental disorders being the most frequent diagnosis (25%), followed by unspecified non-organic psychosis (20%) (table 1 and supplementary table 3). The average number of CNVs per participant was 12.5 (7.4 deletions and 5.5 duplications). Analysis of mean CNV size revealed that pathogenic CNVs were the largest followed by likely pathogenic, and both categories were significantly larger than likely benign and benign CNVs (supplementary figure 1). In line with guidelines of CNV categorisation our results follow the expected size distribution in that pathogenic CNVs are the largest.

Table 1: Descriptive summary of GENMID cohort.

	GENMID
Demographics	
N	599
Ratio (Male/Female)	1.7 (376/223)
Mean age (std.dev)	43.2 (14.1)
ID Level	
Mild	49.2%
Severe	50.8%
Psychiatric diagnoses	
Average number of co-morbid diagnoses (range)	1.6 (1-5)
F84 Pervasive developmental disorders	148 (25%)
F29 Unspecified nonorganic psychosis	121 (20%)
F61 Mixed and other personality disorders	108 (18%)
Challenging behaviours	95 (16%)

F32 Depressive episode	86 (14%)
F41 Other anxiety disorders	60 (10%)
F20 Schizophrenia	49 (8%)
F31 Bipolar affective disorder	47 (8%)
F90 Hyperkinetic disorders	41 (7%)
F42 Obsessive-compulsive disorder	37 (6%)
F43 Reaction to severe stress and adjustment disorders	27 (5%)
F39 Unspecified mood disorder	25 (4%)

Neurodevelopmental CNV frequency analysis

In our sample, we found CNVs in 23 of the 63 NDD loci described by Rees *et al.* (7). At these 23 loci we identified 58 CNV carriers, with two subjects carrying two risk CNVs. The rate percentage in our sample (rate of participants with a NDD CNV) is 10.0%, while the rate percentage is 6.5% in ID/ASD, 3.1% in schizophrenia and 1.2% in healthy control populations (table 2). The NDD loci frequencies are most comparable with the ID/ASD population, a sample which consisted mainly of children with DD/ID and/or ASD (6,7). However, we still observe significantly higher proportions of NDD CNVs in our ID and co-morbid psychiatric diagnosis sample, 3.5% higher (95% CI = 1—6, P = 0.00084).

Table 2: Rate (%) of CNVs at 63 neurodevelopmental disorder (NDD) risk loci in GENMID compared with populations rates reported by Rees et al. 2016. Rate percentage differences, 95% confidence intervals (CI) and p-values for rate comparisons are indicated.

Sample	Sample size	Rate (%) of the 63 NDD-loci	Rate (%) difference (95% CI)	p-value
Healthy control	26628	1.2	8.8 (6.3-11)	2.8e-72
Schizophrenia	20403	3.1	7 (4.5-9.5)	9.7e-21
ID/ASD	29085	6.5	3.5 (1-6)	8.4e-04
GENMID	599	10.0	-	-

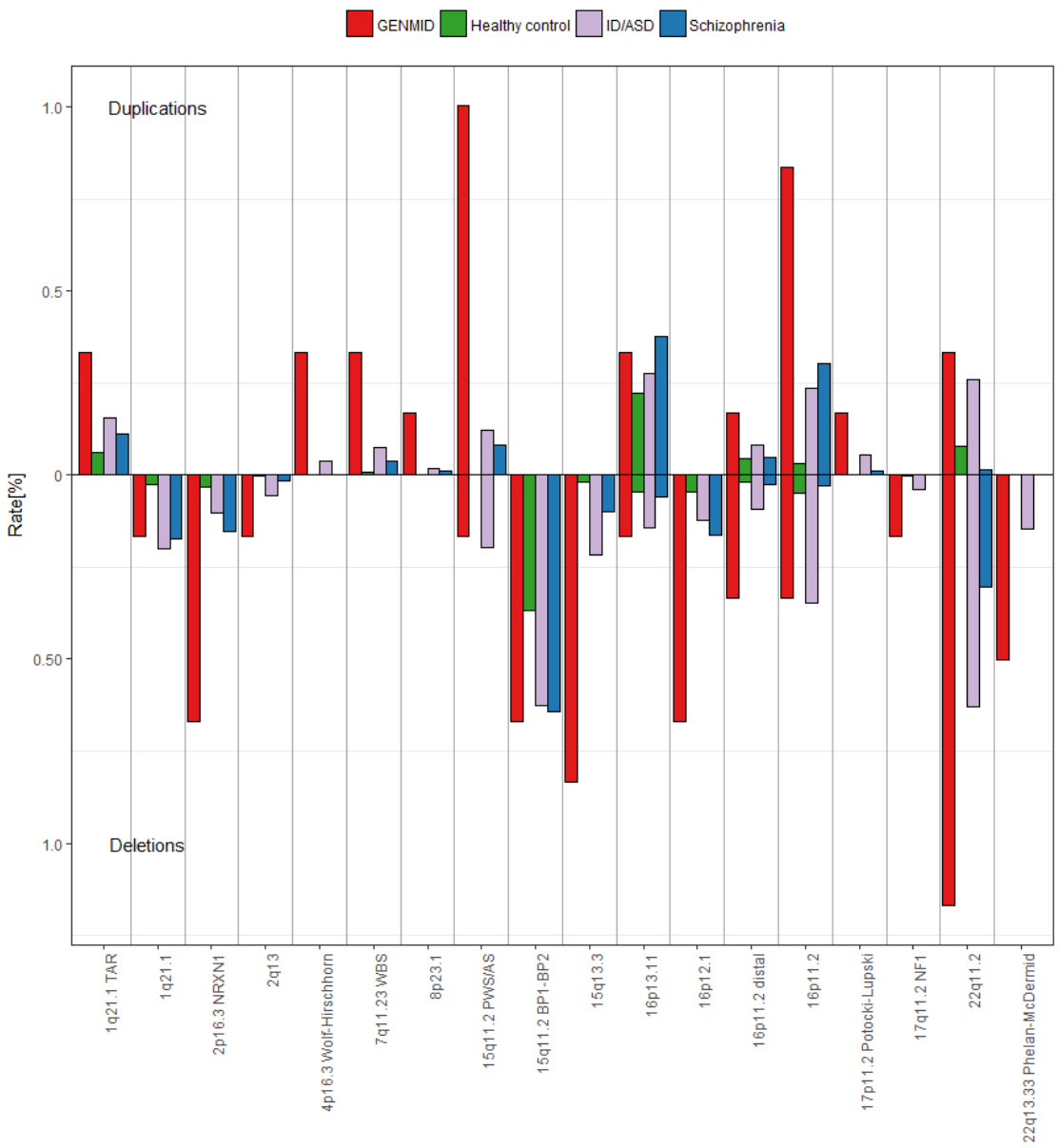

Figure 1: NDD CNV frequencies in the GENMID sample compared to frequencies in healthy controls (N = 26628), ID/ASD (N = 29085) and schizophrenia (N = 20403) cohorts as reported by Rees et al. (7). Rates for deletions extend down from the central line and duplications extend upwards.

The frequencies of the 23 NDD CNVs identified in this cohort are shown in figure 1. The carrier frequency at each loci was the highest in our sample of ID and co-morbid mental illness, with the exception of four loci for which we see comparable frequencies to the ID/ASD cohort. The five most frequent NDD CNVs in the GENMID cohort, in order of frequency, are: 22q11.2 del (N=7, 1.2%), 15q11.2 Prader—Willi syndrome/Angelman

syndrome (PWS/AS) dup (N=6, 1%), 16p11.2 dup (N=5, 0.8%), 15q13.3 del (N=5, 0.8%) and 16p12.1 del (N=4, 0.7%). A description of all CNV loci and the carrier phenotypes can be found in supplementary table 5.

Pathogenic CNV yield

In the GENMID sample 78 participants (13.0%, 95% CI 10.5-16.0) had at least one pathogenic CNV, with similar yields found at all research sites (Catalonia: 13.8%, Leuven: 14.0%, and England: 11.7%). Pathogenic CNVs comprised those identified at the NDD loci previously described and a further 25 CNVs reported as pathogenic by the clinical laboratory services, see supplementary table 6. The pathogenic CNVs were predominantly deletions (59.5%). We previously reported a rate of 11% pathogenic CNVs in a subset of 202 of the 247 participants from the England sample (17). When these 202 individuals are removed from the combined sample the diagnostic yield is 13.9%, thus replicating the initial finding.

ID level, psychiatric diagnoses and CNV pathogenicity

We examined group differences between CNV pathogenicity, psychiatric diagnoses and level of ID. We observe some differences in the proportions of ID and psychiatric diagnoses between the CNV pathogenicity groups (pathogenic, likely pathogenic, likely benign and benign; supplementary figure 2). However, no simple unidirectional relationships were observed. Equally, minor differences in the severity of ID were found between CNV pathogenicity groups, but no overall unidirectional relationship was observed.

Likely pathogenic CNVs

The yield of likely pathogenic CNVs in the sample was 21.5% (95% CI 18.4-25.1). Investigation of recurrent likely pathogenic CNVs revealed 34 CNVs in 16 regions (supplementary table 4). Four recurrent CNVs identified here corroborate existing evidence for the involvement of these regions in neurodevelopmental risk (figure 2).

Figure 2: The locations of four overlapping likely pathogenic CNVs in the GENMID cohort that are implicated in neurodevelopmental disorders in the literature (UCSC genome browser).

First, we identified two carriers of exonic duplications in the *CNTN6* gene at 3p26.3. The CNTN proteins belong to an immunoglobulin super family of cell adhesion molecules and have an important role in neurodevelopmental processes (18). *CNTN6* duplications were first identified in patients with ASD (19,20) and later in a patient with ID and facial dysmorphisms (21). A review of 14 patients with *CNTN6* CNVs revealed that both CNV deletions and duplications affecting *CNTN6* are thought to be involved in variable neuropsychiatric phenotypes (22). The participants identified in our study both presented with mild ID. One had schizophrenia and personality disorder, and one had challenging behaviours and had been convicted of a serious criminal offence. Interestingly, the participant with schizophrenia and personality disorder also had a duplication in the *CNTN4* gene. CNVs affecting *CNTN4* are also thought to confer risk for various NDDs (23).

Second, we identified two participants with CNV duplications at the 9q21.32q21.33 locus encompassing the *SLC28A3* and *NTRK2* genes. SLC28A3 is a nucleoside transporter

involved in the regulation of multiple processes, including neurotransmission; however, there are no prior reports of its role in psychiatric risk. NTRK2 is a receptor tyrosine kinase with numerous neurodevelopmental functions, including synapse formation and plasticity. Altered *NTRK2* expression has been identified in the brains of patients with schizophrenia (24). One participant had severe ID and bipolar disorder (BPD), and the other had mild ID with unspecified non-organic psychosis.

Third, we identified five participants with exonic CNVs in the *CHD* gene family. The CHD proteins are involved in chromatin structure remodeling and the epigenetic regulation of transcription. Three of the participants had exonic CNVs (2 duplications, 1 deletion) in the *CHD8* gene at 14q11.2, which also encompass *SUPT16H*. The protein encoded by the *SUPT16H* gene is thought to be involved in DNA replication and repair. CNV deletions affecting *CHD8* and *SUPT16H* were initially described in children with DD and dysmorphic features (25). Variants in the *CHD8* gene are thought to confer a phenotypic subtype of ASD, comprising macrocephaly, facial dysmorphologies and gastrointestinal abnormalities (26). Both deletions (27) and duplications (28,29) affecting *CHD8* and *SUPT16H* have been described with variable neurodevelopmental phenotypes. The two participants with CNV duplications both had severe ID, one was diagnosed with schizophrenia and the other with BPD. The participant with the CNV deletion also had severe ID and ASD.

Last, we identified two participants with exonic CNVs in the CHD2 gene at 15q26.1 (one deletion and one duplication). Several patients have been described with CHD2 deletions; with a common phenotype of ID, epilepsy, and aggressive challenging behaviours (30,31). To our knowledge, a CNV duplication in CHD2 has not previously been described in the literature. The deletion carrier had severe ID and schizoaffective disorder, and the duplication carrier had challenging behaviours and BPD. Both patients also had an epilepsy phenotype.

Discussion

There is a paucity of research on CNVs in adults with ID and co-morbid psychiatric phenotypes. This poses a challenge for genetic counselling of novel and rare CNVs, as descriptions of later-life phenotypes are largely unavailable. Previous investigations in this patient group identified a diagnostic yield of 11% CNVs classed as clinically relevant (17). In this study, utilising data from three European research sites, we replicate this finding with a higher diagnostic yield of 13.0% pathogenic CNVs in 599 participants (or 13.9% with the previously reported cases removed). Given that CMA is being advocated for use in schizophrenia cohorts, in which the diagnostic yields are lower (between 2.5—5%) (32,33), adults with ID presenting to psychiatric services appear to be a group to prioritise for CMA.

We found CNV carriers at 23 out of the 63 NDD loci. It is unsurprising that we didn't find carriers in the remaining 40 loci, as these CNVs are very rare with reported frequencies in ID between 0.01—0.26% (mean = 0.06%) (7). Presuming that there is an additive effect of having both ID and a co-morbid psychiatric disorder, then we would expect to see an increased frequency of the 63 NDD CNVs in our cohort. Indeed, the cumulative frequency was significantly higher, as compared to both ID/ASD populations not selected for psychiatric co-morbidity and individuals with schizophrenia.

The phenotypic presentation of the NDD CNV carriers is highly variable, both in terms of the level of ID and the psychiatric diagnoses. This indicates a broader role for genes within these CNV loci in conferring general, as opposed to disorder specific, psychiatric risk. It is possible that this clinical heterogeneity partly reflects the difficulty of diagnosing psychiatric disorders in individuals with ID. Interestingly, at least one CNV carrier at each of the five most frequent loci has a psychosis phenotype. Of particular interest are the four carriers of the 16p12.1 deletion, which was significantly associated with risk for schizophrenia by Rees *et al.* (7). Three of the four carriers had a psychosis phenotype, offering further support for this locus as a risk factor for both ID and psychotic disorders.

Second, we determined the diagnostic yield of CMA in our cohort. In addition to the CNVs identified at known NDD loci, we identified a further 25 CNVs that were reported as

pathogenic by the clinical genetic services. The majority of these were large deletion CNVs (1.7Mb—13.2Mb), which overlapped CNVs described in single case reports in the existing literature. This group of CNVs are likely to be extremely rare and thus would not be observed at high enough frequencies in existing case—control studies. We were unable to identify any clear relationship between ID level, co-morbid psychiatric diagnoses and CNV pathogenicity level. This may indicate that NDD CNVs generally have pleiotropic effects, however research with larger sample sizes would be required to further investigate this.

Last, we investigated likely pathogenic CNVs of uncertain clinical significance. Following a literature review of likely pathogenic CNVs that recur in our sample, we were able to offer further support for the involvement of particular CNV regions in neurodevelopmental and psychiatric risk. Unlike the pathogenic CNVs, many of the likely pathogenic CNVs were small (<1Mb) and affected only a small number of genes. There is a growing body of literature for the role of the *CNTN* and *CHD* gene families in risk for ID and co-morbid psychiatric disorders. Again, the phenotypes associated with CNVs affecting these genes appear to be highly variable. It is important to consider the clinical implications of these CNVs, which were not initially reported as pathogenic by the clinical genetics services.

Our findings suggest that if CMA is to be offered more widely to adults with ID presenting with mental disorders many would receive a new genetic diagnosis. Such diagnoses have many implications. They provide, at least, a partial explanation for the person's physical and mental health problems, which in turn may have an effect on illness related beliefs of patients, their families and healthcare professionals. For some CNVs medical and psychiatric associations across the life course are now well described, with implications for clinical management. For example, the 22q11.2 deletion has recommendations for physical health screening, include cardiac, renal and immunology investigations, and psychiatric screening (34). Whilst clinical recommendations for some pathogenic CNVs are less clear, it is important to note that 70% of the CNVs we identified were in NDD risk loci, for which there are existing scientific literature and/or clinical disorder guides available for families and clinicians (35). Identification of pathogenic CNVs also has broader implications for family members, including cascade testing, provision of recurrence risk information and access to support groups. Given the rapid progress of genomic medicine there is a need for

research to address questions of clinical utility and adverse outcomes in order to optimise the process of genetic investigation in ID psychiatry.

One of the limitations of this study is that there were some differences between the populations recruited at the different sites, for example participants were recruited from in-patient psychiatric services in Leuven and outpatient services in Catalonia and England. Most individuals lacked inheritance data, which is a valuable aid in categorisation of rare variants and may have led to an under estimate of our yield. Different platforms were utilised to detect the CNVs at the different sites; however, as all the platforms used were high resolution this is unlikely to have major effects. Finally, a true estimate of the association between CNV pathogenicity and NDD phenotypes would require much larger case-control samples or epidemiological based studies.

This, to our knowledge, is the first large multi-population study of CNVs in idiopathic ID with co-morbid psychiatric disorders. We detected a 13% rate of undiagnosed pathogenic CNVs. From a research perspective, studying this population revealed the highest rate of CNVs at established NDD loci and recurrent likely pathogenic CNVs, both offering unique opportunities for further phenotyping of rare variants. Increased clinical testing and research in this population should be a priority for both clinicians and researchers in the field of psychiatric genetics.

Author contributons

Dr Johan H Thygesen and Ms Kate Wolfe, were the main analysts, participated in all analyses and drafted/revised the manuscript. Dr Marina Viñas-Jornet, Dr Neus Baena, Dr Susanna Esteba-Castillo, Dr Elisabeth Gabau, Ms Nuria Ribas-Vidal, Dr Anna Ruiz, Dr Ramon Novell and Dr Miriam Guitart Feliubadaló, oversaw collection and performed initial analysis of the samples from Catalonia, Spain. Dr Nathalie Brison, Prof Joris Vermeesch, Dr Eddy Weyts, Prof Griet Van Buggenhout and Prof Annick Vogels, oversaw collection and performed initial analysis of the samples from Leuven, Belgium. Dr Andrew McQuillin, Dr Nick Bass, Dr Andre Strydom, oversaw collection and preformed initial analysis of the samples from London, United Kingdom. All authors contributed to conception, design and interpretation of the data, revised the draft and approved the final version to be published.

Declaration of interest

The authors report no conflict of interest.

Previous presentation

This work has not been presented previously, but a subset of data from 202 samples included here has previously been presented in Wolfe *et al.* 2016, four cases in Vogels *et al.* 2014, and one case in each of Vanmarsenille *et al.* 2014, Denayer *et al.* 2012 and Hannes *et al.* 2009.

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Ethical approval

Approval in England was granted by the North Wales Research Ethics Committee West, reference 11/WA/0370. In Catalonia approval was granted by Catalonia Corporació Sanitària Parc Taulí Ethics Coomittee reference 2009/582. In Leuven approval was granted by the Commissie Medische Ethiek van de Universitaire Ziekenhuizen KU Leuven, reference S54583 (ML8614).

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